UREA H.P.

(UREASE-CHLORITE) End-Point

BIO LAB DIAGNOSTICS
ISO 9001:2015
ISO 13485:2016
CE

CLINICAL SIGNIFICANCE

Increased levels are associated with renal disease as well as dehydration, diabetic coma, hypoadrenal crisis, gastrointestinal hemorrhage and circulatory collapse. Decreased values are observed in some cases of severe liver diseases.

PRINCIPLE

Urea in presence of urease enzyme separates into NH_3 and Co_2. Ammonia reacts in an alkaline ambience with cresol hypochlorite to form a GREEN compound whose absorbance can be measured at 600 nM.

REAGENTS COMPOSITION

1. UREASE REAGENT

EDTANa	1.24 mMol/L
Urease	20 KU/L

1B CHROMOGEN

Buffer	100 mMol/L
Cresol	3 mMol/L
Nitroprusside	3.5 mMol/L

2. HYPOCHLORITE

Hypochlorite	8 mMol/L
Sodium Hydroxide	140 mMol/L

3. STANDARD

Urea	40 mGs/dL
Urea Nitrogen	18.65 mGs/dL

Working Reagent Preparation

Reconstitute one vial of Enzyme Reagent No. 1 with Reagent No. 1B. Reagent No. 2 and 3 are ready to use.

STORAGE AND STABILITY

Unopened kit is stable until expiry date marked on each label. Reconstituted Reagent No. 1 is stable for 15 days at 2-8°C and for 6 days at (25-30°C).

SAMPLE

Sample can be serum or plasma which has no sign of hemolysis. Avoid anticoagulants having ammonia salt or anticoagulants containing ammonium oxalate. If Urine sample is to be tested it must be diluted 1 to 100 and the result to be multiplied by 100 (dilution factor). (All samples should be handled as potential infective agents as no laboratory methods make conclusive findings for its safety. Therefore, adequate protective laboratory measures should be taken while handling such materials).

PROCEDURE

Pippette into three test tubes labelled as	BLANK	STD	TEST
Working Reagent No. 1 ..... µL	1000	1000	1000
Standard Reagent No.3..... µL	-	10	-
Sample (Serum or Plasma)..... µL	-	-	10
Mix well. Incubate for 6 minutes at 37°C.			
Add Working Reagent No. 2 ..... µL	1000	1000	1000

Mix well. Incubate for 6 minutes at R.T. Read at 600 nM (580-620 nM) against Blank. Final colour is stable upto 30 minutes. Reagent & sample Volumes can be altered proportionately to suit the cuvette size.

NOTE : Programme the analyzer using system parameters. A specific programme data sheet may be provided for each analyzer upon request.

SYSTEM PARAMETERS

Reaction	End-Point
Temperature	37°C
Wavelength	600 nM (580-620 nM)
Standard Concentration	40 mGs/dL
Absorbance Range	0.2 Å
Cuvette Path Length	1 cM
Reagent Volume	**(R_1 1 mL + R_2 1 mL)**
Sample Volume	**10 µL**
Reaction Time	6 + 6 = 12 minutes
Linearity	120 mGs/dL
Max. limit of blank rgt.	0.3 Å
Final Colour Stability	30 mins

RESULTS

Urea in mGs/dL =

$$\frac{\text{OD Test - OD Blank}}{\text{OD Std - OD Blank}} \times 40$$

BUN = Urea × 0.1667 mMo/L

Example:

OD of Blank = 0.04 and OD of Test = 0.35
OD Standard = 0.65

$$\text{Urea in mGs/dL} = \frac{0.35 - 0.04}{0.65 - 0.04} \times 40 = 20.32$$

For values higher than 120 mGs/dL dilute the sample with distilled water free from ammonium salts and rerun the assay. Multiply the results with dilution factor i.e. by 2 for 1:1 dilution.

EXPECTED VALUES

Serum of Plasma Urea	20 - 40 mGs/dL
BUN	9.4 - 18 mGs/dL
Urine Urea	20 - 35 Gms/24hrs.

As with all diagnostic methods, the final diagnosis should not be made on the result of a single test as well as laboratory diagnosis must be confirmed with clinical manifestations.

LIMITATIONS

Introducing contaminated pipettes or chemicals make false results. Enzyme reagent is susceptible to deterioration at room temperature. This assay is linear upto 120 mGs/dL. **High values are observed if the assay reagents or samples are contaminated with ammonia salt or anticoagulants containing ammonium oxalate.**

WARNING

This reagent system is for *INVITRO* use only. This reagent system is containing preservatives and components that have not established for safety if contacted on broken skin or eye or taken orally. In case of such incidents wash off with plenty of water, or consult a physician.

QUALITY CONTROL

To ensure adequate quality control, each kit should be tested against a standard control serum. It should be realized that the use of quality control material checks both instrument and reagent function together. Factors which might affect the performance of this test include proper instrument functions, temperature control, cleanliness of glassware and accuracy of pippetting.
It is appropriate to establish each laboratory's accuracy constant and interpret values. Similarly, laboratory findings should be established by clinical manifestations.

BIBLIOGRAPHY

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BIOLAB DIAGNOSTICS (I) PVT. LTD.
J-245, MIDC, TARAPUR, BOISAR - 401 501, MAHARASHTRA.
E-mail : biolab@vsnl.com / www.biolabdiagnostics.com
Customer Care : (+ 9122) 28088243